CHASSIS

Growth Curve Experiment

Results

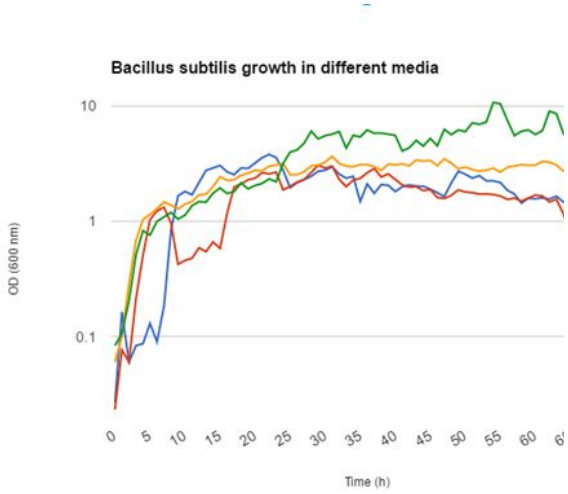

Fig 1. Line graph depicting OD 600nm measurement of *Bacillus subtilis* growth at set time points over 70 hours. Data is compiled as the average of 3 replicates of each experiment. Legend of color lines corresponds to the type of media added during growth experiment. OD600 measurements are labelled on the y-axis while time points are indicated on the x-axis.

Time (h)	Nothing (1)	Nothing (2)	Nothing (3)	LB (1)	LB (2
0	0.02	0.02	0.04	0.04	0.0
1	0.24	0.16	0.09	0.1	0.0
2	0.15	0.02	0.01	0.11	0.0
3	0.1	0.12	0.03	0.28	0.2
4	0.12	0.13	0.01	0.61	0.4
5	0.18	0.11	0.1	1.01	1.0
6	0.11	0.05	0.11	1.33	1.1
7	0.19	0.14	0.22	1.34	1.2
8	0.76	0.775	0.97	0.865	0.79
9	1.68	1.64	1.65	0.05	0.0
10	1.66	1.84	1.92	0.01	0.0
11	0.79	1.96	2.32	0.04	0.0
12	1.87	2.21	2.41	0.01	0.
13	2.82	2.74	2.69	0.03	

14	2.44	3.09	3.13	0.06	0.0
15	2.71	3.17	3.22	0.05	0.0
16	2.335	2.8	2.895	1.01	0.67
17	2.45	2.47	2.67	2.25	1.
18	2.78	2.85	3.02	2.36	1.8
19	2.73	2.86	2.98	2.49	1.9
20	3.08	3.23	3.26	2.63	2.0
21	3.65	3.41	3.56	2.78	2.4
22	3.83	3.73	3.79	2.92	2.1
23	3.63	3.53	3.5	3.12	2.2
24	2.98	2.26	3.02	2.33	1.95
25	1.99	1.3	2.55	2.1	1.9
26	2.3	1.57	2.6	2.34	2.0
27	2.39	1.63	2.83	2.39	2.2
28	2.65	1.86	2.83	3	2.4
29	2.96	2.22	2.92	3.52	2.
30	3.13	2.26	2.89	3.22	2.6
31	3.4	2.36	3.1	3.2	2.9
32	3.045	2.465	2.16	2.365	1.95
33	2.45	2.38	2.24	1.95	1.5
34	2.74	2.38	2.21	2.28	1.9
35	0.236	2.22	1.98	2.58	2.1
36	2.23	2.11	1.96	2.78	2.5
37	2.16	2.04	1.02	3.17	2.7
38	2.18	2.15	1.89	2.91	2.2
39	2.09	2.32	1.72	3.12	2.5
40	1.775	1.795	1.845	2.58	2.3
41	2.02	2	1.93	2.09	2.0
42	1.99	2.21	1.97	1.99	2.0
43	2.11	2.08	1.84	2.03	2.0
44	1.99	2.24	1.79	1.91	1.8
45	1.93	1.91	1.83	1.97	1.8
46	1.69	1.9	1.7	1.62	1.6

47	1.71	1.62	1.57	1.71	1.4
48	2.17	2.225	2.02	1.965	1.
49	2.7	2.88	2.53	2.48	1.6
50	2.5	2.7	2.44	2.43	1.5
51	2.34	2.49	2.24	2.34	1.5
52	2.49	2.6	2.34	2.22	1.5
53	2.15	2.28	2.28	2.14	1.5
54	2.2	2.28	2.21	2.15	1.5
55	2.07	2.25	2.16	2.22	1.4
56	1.87	1.89	1.795	1.885	1.6
57	1.73	1.7	1.72	1.85	2.0
58	1.45	1.53	1.32	1.55	2.0
59	1.71	1.71	1.31	1.68	2.
60	1.65	1.52	1.52	1.72	2.4
61	1.67	1.57	1.53	1.72	2.3
62	1.64	1.54	1.48	1.62	2.0
63	1.63	1.61	1.69	1.55	2.2
64	1.455	1.41	1.515	1.2	1.4
65	1.38	1.49	1.75	0.83	0.8
66	1.35	1.46	1.52	0.83	0.8
67	1.19	1.37	1.51	0.89	0.
68	1.21	1.2	1.48	0.87	0.
69	1.12	1.33	1.5	1	0.9
70	1.32	1.26	1.59	0.88	0.8
71	1.18	1.25	1.52	0.81	0.
72	1.16	1.2	1.47	0.99	0.9

Table 1. Raw data of the growth experiment of *Bacillus subtilis* by measuring growth as a function of OD 600nm readings with its respective time points.

Fig 2. Line diagram depicting the growth pattern of *Bacillus subtilis* when subjected to differing temperatures, using OD600nm spectrophotometry as a method to measure cell growth over time. The data points depicted in this diagram are averages of data collected over 3 replicates for each experiment, shown in Table 2. Time is modelled on the x-axis whereas OD600 is governed by the y-axis.

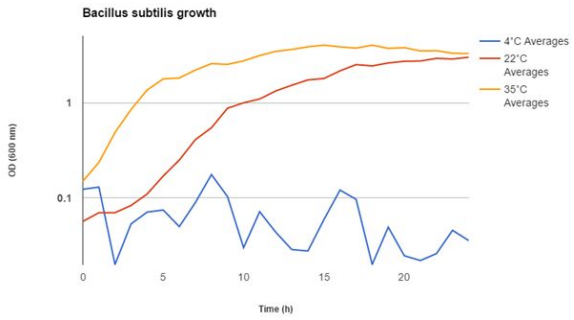

Fig 2. Line diagram depicting the growth pattern of *Bacillus subtilis* when subjected to differing temperatures, using OD600nm spectrophotometry as a method to measure cell growth over time. The data points depicted in this diagram are averages of data collected over 3 replicates for each experiment, shown in Table 2. Time is modelled on the x-axis whereas OD600 is governed by the y-axis.

Time (h)	4˚C (1)	4˚C (2)	4˚C (3)	22˚C (1)	22˚C (2)
0	0.29	0.03	0.05	0.1	0.04
1	0.34	0.02	0.03	0.05	0.08
2	0.06	0	0	0.07	0.09
3	0.16	0	0	0.08	0.11
4	0.09	0.063	0.06	0.1	0.13
5	0.05	0.055	0.119	0.14	0.19
6	0.02	0.059	0.071	0.2	0.3
7	0.14	0.064	0.064	0.36	0.5
8	0.4	0.058	0.07	0.47	0.595
9	0.31	0	0	0.89	0.76
10	0.09	0	0	0.84	1.03
11	0.17	0	0.045	0.84	1.07

12	0.05	0.039	0.042	1	1.46
13	0.01	0.038	0.038	0.93	1.74
14	0	0.043	0.04	1.08	2.08
15	0.09	0.042	0.046	1.2	1.93
16	0.285	0.037	0.041	1.48	2.545
17	0.29	0	0	1.84	2.76
18	0.06	0	0	1.6	2.95
19	0.06	0.049	0.039	1.67	3.25
20	0.01	0.032	0.032	1.76	3.33
21	0	0.033	0.033	1.82	3.26
22	0	0.041	0.037	2.01	3.55
23	0.06	0.038	0.039	1.71	3.63
24	0.03	0.034	0.043	2.05	3.59

Table 2. Raw OD 600nm data of *Bacillus subtilis* growth curve experiment as described in the protocol available on the wiki. Differing temperature and time controls are indicated.

Interpretation

In regards to the media addition growth experiment, there are several points in which it would be important to address. There was little difference between the addition of LB and the control of no media addition. This is an unexpected result, as one would expect the nutrient in the media to be used over time by the cells and need to be replaced by the addition of new media; no further media added was expected to slow down growth of the bacteria. However, it seems as though this effect was minimal and that the addition of LB proved to have little effect on the growth of the cells. Another interesting feature of this experiment was that the addition of super rich (SR) far surpassed all other media addition treatments. For example at the final time point (70-hour),

the SR treatment had an OD600nm average of 8.2 whereas the closest treatment (being the 2-times concentrated LB media) had only an OD600nm average of 3.65. This is a clear indication that the SR media has the nutrient concentration most ideally suited for *B. subtilis* growth, and would be a suitable candidate for use in our patch.

There are several conclusions which can be drawn from the temperature growth curve experiment. One, that at the ambient temperature of the international space station (22C), *B. subtilis* grows to a similar OD600 as the ambient skin temperature (35C) nearing the 24-hour mark. However, it grows much slower before the end of the experiment; at the 10-hour mark the OD600 average of the 22C treatment was 1.00 whereas the OD 600 average of the 35C treatment was 2.76. This is an interesting phenomenon, as it is likely that by the 24-hour time point the 35C treatment cells had used up most of the nutrient in their media, and this slowed growth immensely. On the other hand, the 22C cells had not grown near as much as the 35C cells as such would likely still have much of their nutrient in media still remaining. This is most likely the reason as to why these data points seem to converge at the 24-hour time point. The 4C treatment was always far below the other temperature treatments, never reaching over 0.4 OD600. This suggests that at 35C, the cells will be able to grow at their optimal (from what we determined), and as such our skin should suffice to keep the cells warm enough to grow properly.

Project Achievements

Found that the temperature of 35C (skin temp) is a good temperature to grow *B. subtilis* at, while super rich media is the best media to use for growth.

Future Plans

None. Complete!

Transformation and Isolation of pSB1c3 containing BBI-5 GFP in *E. coli* TOP 10

Results

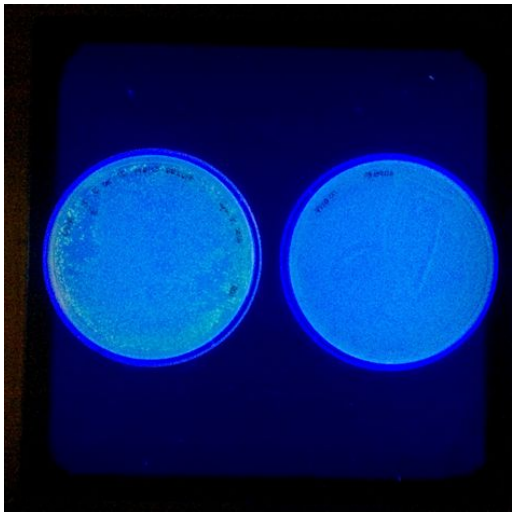

Fig 3. Photograph of TOP10 *E. coli* colonies on petri dishes transformed with (left plates) and without (right plate) pSB1c3 GFP-BBI5 when viewed under UV light.

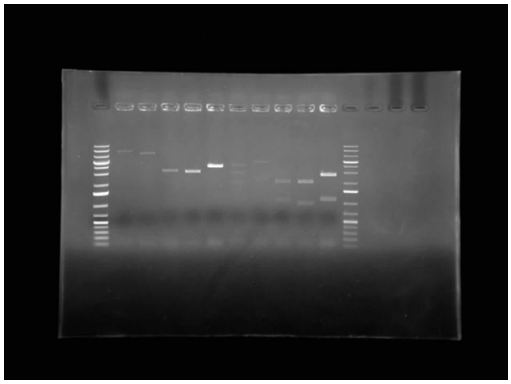

Fig 4. 1% agarose gel electrophoresis to confirm ligation of BBI-GFP constructs in *E. coli* chassis. Lanes: 1. 1 Kb Plus GeneRuler DNA Ladder, 2. pMLK83 digested with Xba1 3. pSG1154 digested with EcoR1, 4. pSB1c3-RFP digested with EcoR1, 5. pSB1c3-BBIGFP digested with EcoR1, 6. pSB3c5-152001 digested with EcoR1, 7. pMLK83 digested with EcoRI + PstI, 8. pSG1154 digested with EcoRI + PstI, 9. pSB1c3-RFP digested with EcoRI + PstI, 10. pSB1c3-BBI5GFP digested with EcoRI + Pst, 11. pSB3c5-I52001 digested with EcoRI + PstI, 12. 1 Kb Plus GeneRuler DNA ladder.

Interpretation

While subjective evidence is the least credible scientific evidence, this is a clear indication that the pSB1c3 GFP-BBI5 vector was successfully transformed into *E. coli* when compared to the untransformed control (right plates on Fig. 3). The transformed plate is clearly glowing with green fluorescence, whereas the untransformed plate's *E. coli* colonies clearly aren't. Interestingly, this also proves that the GFP-BBI5 fusion does not have a visible effect on the function of GFP; this is important for further tests when using the GFP-BBI5 fusion.

After restriction digest and ligation of the respective plasmids with their constructs (as defined in the lanes of Fig. 3), *E. coli* strain TOP10 samples were transformed with the ligated products. These samples were mini-prepped to isolate the plasmids, then ran on a 1% agarose gel. This gel electrophoresis confirms several of our construct plasmids due to the expected size matching with the sizes observed on the gel (Fig.3). The unligated plasmids (the controls) all adhered to their expected sizes, for example pMLK 83 digested by Xba1 (lane 2) had an expected size of 9.9kb and ran at 9.9kb on the gel. pSG1154 digested with EcoR1 (lane 3) had an expected size of 7.6 kb and was observed at 7.6 kb on the gel. This improves the confidence with which we move forward in this experiment. Most interesting in terms of our project are the BBI constructs we made. pSB1c3-BBI5GFP (lane 5) and pSB1c3-BBI5GFP (lane 10) both show their expected sizes matching the sizes found on the gel. This suggests that our restriction digest and ligation of the BBIGFP constructs worked to insert the construct into its respective plasmid.

Project Achievements

GFP-BBI5 transformed into *E. coli*, and was successfully isolated as a plasmid. We can now take this pSB1c3 plasmid and transform *B. subtilis* with this plasmid.

Future Plans

GFP-BBI5 transformed into *E. coli*, and was successfully isolated as a plasmid. We can now take this pSB1c3 plasmid and transform *B. subtilis* with this plasmid.

Isolation of pSB1c3 containing Comk with and without Amy E homology sites in *E. coli* TOP 10

Results

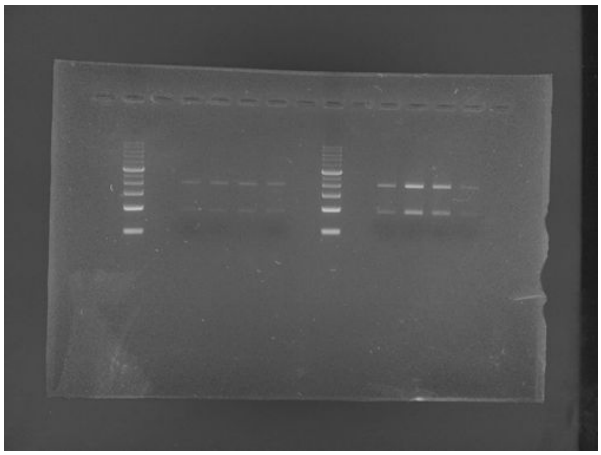

Fig 4. 1% gel electrophoresis to confirm pSB1c3 GFP-BBI5 construct and pSB1c3 ComK construct grown in *E. coli* chassis. All plasmids were digested with EcoR1 and Pst1. Lanes 2 and 9 are the Plus GeneRuler DNA ladder. Lanes 4-7 are pSB1c3-GFP-BBI5 construct, lanes 11-14 are the pSB1c3 ComK construct.

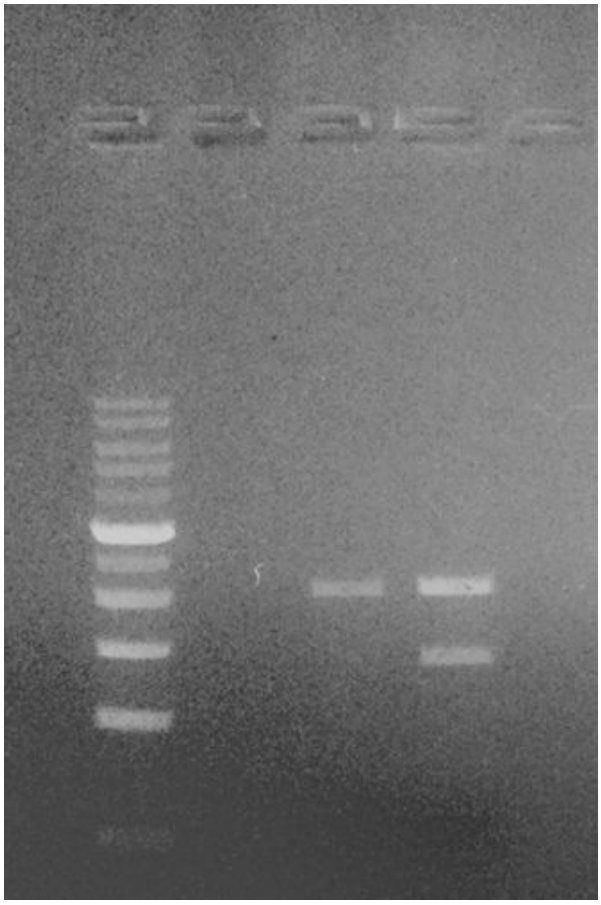

Fig 5. 1% gel electrophoresis to confirm pSB1c3-ComK with AmyE homology regions. Lanes: 1. GeneRuler DNA ladder. 2. Empty 3. pSB1c3 (no insert) 4. pSB1c3 with AmyE regions, digested by EcoR1 and Pst1.

Interpretation

This confirmation ensured that the digestion and ligation of GFP-BBI5 and ComK were correctly inputted into the plasmids that we needed for further transformation into *Bacillus subtilis*. The expected band size of pSB1c3 is 2062 bp, while GFP-BBI5's is 933 bp and ComK's size is 966 bp. All of these expected sizes match what is seen on the gel.

Project Achievements

This is another step in the transformation of *B. subtilis* with ComK

Future Plans

Now that the pSB1c3 plasmid has confirmed to have ComK ligated within it, the plasmid has also been isolated. *Bacillus subtilis* can now be

transformed with the purified plasmid. Further, now that the ComK sites with AmyE homology sites have been confirmed as well, it is possible to transform *Bacillus subtilis* and then integrate the plasmid into the chromosome due to the presence of the homology sites.

Sequencing data obtained from sequencing pSB1c3 with ComK with AmyE site from *E. coli* TOP 10

Results

NNNNNNNNTTNNNNTATANNAAATAGGCGTATCACGAG
GCAGAATTTCAGATAAAAAAAATCCTTAGCTTTCGCTANGA
TGATTTCTGGAATTCACCATAATGGGGTATATGGTTTCTGG
TTATAATCCATATCAGATTGCTGAAGTTCTGGATATGGATA
TCCGTAGCATCTACGCGTACAAGCAACGAATCGAAAAGAG
AATGGGTGGTAAAATAAACGAATTATTTATTCGTTCACATT
CGGTCCAACATTGATACTCAACGACCAGCCAGAATCATACT
CTGTTTATACGGGCATTTATTGAAATGTCCGTTTTTATAAAT
AGGCCGTCCCCTCAAGAGTAAACACCATAGTATTTAAAACC
ATCACACGAAAAATTCAGAAGCATTACGAAATATGAATTTA
CAAACAATAGTGGCATAAATGTTAACCATGTTAATTTACGT
AAAGTTTTACGTTGCAACATTAAAGCCTCATTTCAATCATCA
TGATAAATATAAAATTAATATATATTTATGCCGTAAATACCA
ATATACTTAGCAAACTATGTGATCTCCATTTCGATTGATTTA
GTGTTTATTGACGTATGTACTGGATTATTAACGATAATATC
GAGTTCTGGCCTGAGCACCGAAAATTAATATCGGTACATAA
CGCCGATCTTAACGTCGTTCTGACAACGCCAGCCAGTCGAT
GTTTATCACTTCTACTTGAAGCTTTTCCTGATGTGGTTGCAC
AACAAGATTTTTTCACCAGAGTCTGGGAAGAAGAAGGTAT
GCGTGTGCCTACTAACACGTTATATCAGAACATATCCATTA
TCAGACGCGGATTTCGCGCTGTTGGTGATACTACCCACTCG
CTAATTGCAACCGTGCCGAGAAGAGGATTCAAGATCCATA
ATGACATTAACATACAAAATCATGTAATAAACTCGTCAACA
GACGCACATACACACAATGCCCCACCTGCCATAAAAGTTAA
TGCGGNTACAAAGAGAGCATTGGNGGCGCAAAGAATTTC
AATAACAAAATCCTCAAACNNATAAANTCATCTAATNNNG
TTGAGCGCATTTGTCATNGNNNNTATTCTGCN

Fig 6. Sequencing data obtained from sequencing pSB1c3-ComK with AmyE site.

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#=======================================
#
# Aligned_sequences: 2
# 1: EMBOSS_001
# 2: EMBOSS_001
# Matrix: EDNAFULL
# Gap_penalty: 16
# Extend_penalty: 4
#
# Length: 170
# Identity:      96/170 (56.5%)
# Similarity:    96/170 (56.5%)
# Gaps:          15/170 ( 8.8%)
# Score: 88
#
#
#=======================================

EMBOSS_001       391 ATTACGAAATATGAATTTACAAACAATAGTGGCATAAATGTTAACCATG
                     ||.||.||||.|   |.||.|||.||.|.||..|||||..||.|...||
EMBOSS_001       139 ATAACTAAATTT---TCTAAAAAAAACATTGAAATAAACATTTATTTTG

EMBOSS_001       441 TAAT--TTACGTAAAGTTT-TACGTTGCAACATTAAAGCCTCATTTCAA
                     ..||  |.|..|||||||. |...|||.|..|..|||  ||.|.| |||
EMBOSS_001       186 ATATGATGAGATAAAGTTAGTTTATTGGATAAACAAA--CTAACT-CAA

EMBOSS_001       488 CATCATGATAAAT--ATAAAATTAATA---TATATTTATGCCGTAAAT-
                     .|..||..|..||  |||||.||..|.   ||.||..|.|..|.|||| 
EMBOSS_001       233 TAAGATAGTTGATGGATAAACTTGTTCACTTAAATCAAAGGGGGAAATG

EMBOSS_001       532 CCAATATACTTAGCAAACTA    551
                     |.|||...|..||..|.|||
EMBOSS_001       283 CAAATGGTCCAAGATATCTA    302


#---------------------------------------
#---------------------------------------
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Fig 7. Sequence comparison between the sequenced ComK gene and the expected ComK gene, showing 56.5% consensus between the sequences

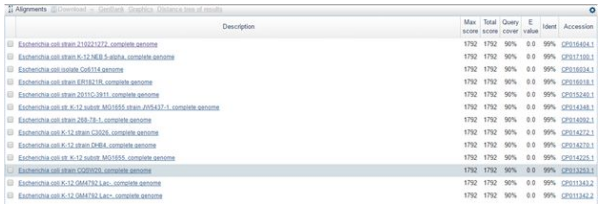

Fig 8. BLAST run of sequenced ComK gene displaying high consensus regions between the sequenced gene and *E. coli* chromosomal sequences.

Interpretation

This result was not the expected result from the sequencing information. The 56.5% consensus sequence suggests that there is little consensus between the expected and actual sequences of ComK. Furthermore, there is a high sequence homology between the sequenced gene and several parts of the *E. coli* chromosome. From the sequence, it appears as though chromosomal sequences from *E. coli* had somehow managed to make it into the pSB1c3 plasmid. There are a variety of methods unto which this result could have taken place, but the

most reasonable conclusion that one can draw from this data is that a chromosomal-plasmid recombination event had taken place, adding in parts of the *E. coli* chromosome.

Project Achievements

This does not confirm that pSB1c3 has been isolated successfully. This would be enough to submit a part.

Future Plans

This is the end of the experiment for this particular piece, however. In the future, we would need to try to re-ligate ComK with flanking AmyE sites into the pSB1c3 plasmid and re-start the experiment, and hopefully no recombination event takes place that inserts *E. coli* DNA into the pSB1c3

Sequencing data obtained from sequencing pSB1c3 with ComK with AmyE site from *B. subtilis* WB800

Results

NANNNNNNTTNANCCTATAAAAATAGGCGTATCACGAGGCAG
AATTTCAGATAAAAAAAATCCTTAGCTTTCGCTAAGGATGATTTC
TGGAATTCGCGGCCGCTTCTAGAGAATTTTGTCAAAATAATTTTA
TTGACAACGTCTTATTAACGTTGATATAATTTAAATTTTATTTGAC
AAAAATGGGCTCGTGTTGTACAATAAATGTATATTAAGAGGAG
GAGATATATATAATGAATATCAAGAAGTTCGCAAAACAGGCGA
CAGTCCTGACCTTTACCACCGCCTCTTGGCAGGGGGGGCGACCC
AGGCATTCGCTGCTTGTTCTTCTTCCCCATCTAAGCATTGTGGTA
AATCATGCATTTGCGCACTGTCATATCCGGCACAATGCTTTCGTA
AAGGAGAAGAACTTTTCACTGGAGTTGTCCCAATTCTTGTTGAA
TTAGATGGTGATGTTAATGGGCACAAATTTTCTGTCAGTGGAGA
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CTACTTTCGGTTATGGTGTTCAATGCTTTGCGAGATACCCAGATC
ATATGAAACAGCATGACTTTTTCAAGAGTGCCATGCCCGAAGGT
TATGTACAGGAAAGAACTATATTTTTCAAAGATGACGGGAACTA
CAAGACACGTGCTGAAGTCAAGTTTGAAGGTGATACCCTTGTTA
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ATTCTTGGACACAAATTGGAATACAACTATAACTCACACAATGTA
TACATCATGGCAGACAAACAAAAGAATGGAATCAAAGTTAACTT
CAAAATTAGACACAACATTGAAGATGGAAGCGTTCAACTAGCA
GACCATTATCAACAAAATACTCCAATTGGCGATGGCCCTGTCCTT
TTACCAGACAACCATTACCTGTCCACACNATCTGCCCTTTCGAAA
GATCCCAACGAAAAGAGAGACCACATGGTCCTTCTNAGTTTGTA
NCAGCTGCTGGGATTACNNNTGGCATGNNGACTA

Fig 9. DNA nucleotide sequence results of pSB1c3 containing BBI5-GFP from *B. subtilis*

Fig 10. Consensus analysis of the expected DNA and the actual DNA obtained from the isolation of pSB1c3 containing BBI5-GFP from *B. subtilis*

Interpretation

The DNA consensus sequences between the expected pSB1c3 containing BBI5-GFP and the actual was quite high, at 86.6% consensus. This means that there is a high probability that *B. subtilis* was in fact transformed with pSB1c3 containing BBI5-GFP.

Project Achievements

This is very significant for our project, as this suggests that *B. subtilis* is susceptible to our plan (modularity).

Future Plans

Next time, it would be prudent to transform *B. subtilis* with a plasmid containing BBI5 without the GFP tag to confirm that this, on its own, can be added to *B. subtilis*.

Mass Spectrometry

Interpretation

The presence of BBI fused with TD-1 was examined in the blood through mass

spectrometry. The blood samples were obtained from the mice sacrificed in the prototype testing study, and three replicates were used for each time point. As mentioned in that study 3 groups were used, and this analysis follows the group that was used to examine BBI-TD1 diffusion across the skin into the blood. Previous iGEM teams have shown that TD-1 works as a transdermal tag, which assists in peptide diffusion across the skin, however, no diffusion was observed in our experiment. The mass spectrometry results were negative, and no BBI-TD1 was found in the blood plasma or blood serum of control or the experimental mice samples. The monoisotopic uncharged mass of 2577.1 was calculated for the assay and the fragment spectra (i.e. MS/MS) for the +3 and +4 charge states at m/z 859.36 and 644.77 are shown in the figure above. The results can be explained partly by protein degradation in body and partly by the hypothetical failure of TD-1 to act as a transdermal tag. Since BBI is a small peptide, it is very likely to get excreted by the kidneys, and since we don't have constant production in the patch it can be presumed that a constant concentration was not achieved in the blood. On the other hand, it can also be presumed that TD-1 may have failed to act as a transdermal tag, so no peptide diffused through the skin.

Project Achievements

Further characterization of BBI
Further characterization of TD-1

Future Plans

The study can be conducted using a different transdermal tag

A shorter timeline can also be used instead of day-2 and day-5 blood sampling. The blood drawing can be done in few hours to avoid degradation, which can also assist in determining the pharmacokinetics of the drug.

The study can be conducted again once the

Bacillus subtilis is transformed with BBI to fully examine the functioning of the prototype.

Gel electrophoresis to confirm BBI ligation into the pSB1c3 plasmid

Results

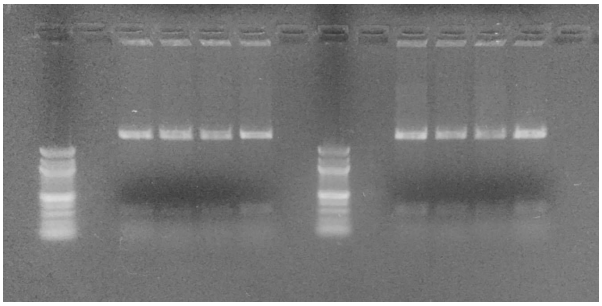

Fig 11. Gel electrophoresis of our BBI construct ligated into a pSB1c3 plasmid. Lanes: 1. Amresco 100bp DNA ladder. 2. Null. 3. BBI Construct 4. BBI Construct 5. BBI Construct 6. BBI Construct 7. Null 8. Amresco 100bp DNA ladder 9. Null 10. BBI Construct 11. BBI Construct 12. BBI Construct 13. BBI Construct 14. Null

Interpretation

The size of BBI's size in DNA bp experimentally determined matched that of the expected size, being around 275 bp. We know that the DNA on the gel ran correctly because the controls of the DNA ladder as well as the null wells (where no DNA was added) displays.

Project Achievements

This is very significant for our project, as BBI is a part that we wish to submit to the registry, and now this is possible.

Future Plans

From here, we would like to be able to transform *B. subtilis* with this plasmid to begin to produce BBI from our chassis, an integral part of our project.

Gel electrophoresis to confirm that KSCI-BBI was ligated into the pSB1c3 plasmid

Results

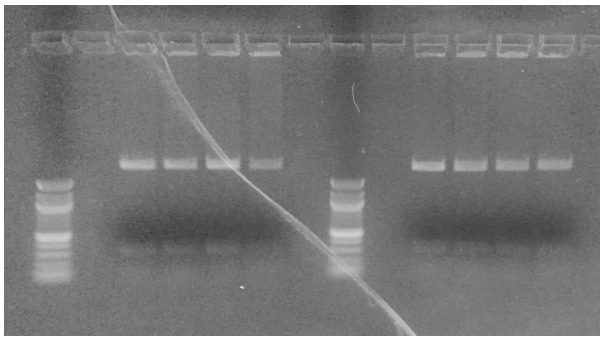

Fig 11. Gel electrophoresis of our BBI construct ligated into a pSB1c3 plasmid. Lanes: 1. Amresco 100bp DNA ladder. 2. Null. 3. KSCI-BBI Construct 4. KSCI-BBI Construct 5. KSCI-BBI Construct 6. KSCI-BBI Construct 7. Null 8. Amresco 100bp DNA ladder 9. Null 10. KSCI-BBI Construct 11. KSCI-BBI Construct 12. KSCI-BBI Construct 13. KSCI-BBI Construct 14. Null

Interpretation

The size of KSCI-BBI's size in DNA bp experimentally determined matched that of the expected size, being around 293 bp. We know that the DNA on the gel ran correctly because the controls of the DNA ladder as well as the null wells (where no DNA was added) displays.

Project Achievements

This is very significant for our project, as KSCI-BBI is a part that we wish to submit to the registry, and now this is possible.

Future Plans

From here, we would like to be able to transform *B. subtilis* with this plasmid to begin to produce KSCI-BBI from our chassis, an integral part of our project. As well, the KSCI-tag would allow our peptide to be soluble in water-based environments (such as human blood).